

Listing of Claims.

Please amend the claims as shown below by deleting the material indicated by strike-through and adding the underlined material. This listing of claims will replace all prior versions and listings of the claims in this application.

1. (Currently amended) A method of evaluating clotting activity in a sample comprising:

(a) combining in vitro a blood or plasma sample from a subject comprising blood or plasma with:

- (i) a soluble phospholipid that is soluble in the sample;
- (ii) a contact activator; and
- (iii) calcium;

(b) incubating the mixture of (a) above for a time and under conditions sufficient for prothrombinthrombin activation; and

(c) detecting Factor X_a or thrombin activity, wherein the activity of Factor X_a or thrombin correlates withis indicative of clotting factor activity in the sample, thereby evaluating clotting activity in the sample.

2. (Original) The method of Claim 1, wherein the sample is from a subject with lupus.

3. (Currently amended) The method of Claim 1, wherein the sample is further combined with Activated Protein C or a Protein C activator, wherein the level of thrombin activity correlates withis indicative of Activated Protein C resistance in the sample.

4. (Currently amended) The method of Claim 3, wherein the sample is further combined with Protein S depleted plasma, wherein the level of thrombin activity inversely correlates withis indicative of Protein S levels in the sample.

5. (Original) The method of Claim 1, wherein the sample is further combined with a plasma selected from the group consisting of (a) plasma known to be deficient for a particular clotting factor and (b) normal plasma.

6. (Original) The method of Claim 1, wherein the sample is from a subject that has been given heparin treatment.

7. (Original) The method of any of Claims 1-6, wherein thrombin enzymatic activity is measured.

8. (Original) The method of any of Claims 1-6, wherein clot formation is detected.

9. (Currently amended) The method of Claim 1, wherein the ~~soluble~~ phospholipid consists essentially of a phospholipid selected from the group consisting of phosphatidylserine, phosphatidylhomoserine, phosphatidic acid, phosphatidylethanolamine, and a combination thereof.

10. (Currently amended) The method of Claim 9, wherein the ~~soluble~~ phospholipid consists essentially of phosphatidylserine acylated by C2 to C14 fatty acids~~C6 phosphatidylserine~~.

11. (Currently amended). The method of Claim 1, wherein the ~~soluble~~ phospholipid is added to a final concentration from about 4 μ M to about 2 mM.

12. (Currently amended) The method of Claim 1, wherein the ~~soluble~~ phospholipid is in a dried form prior to combination with the sample.

13. (Original) The method of Claim 1, wherein the sample is a human blood or plasma sample.

14. (Original) The method of Claim 1, further comprising comparing the detected thrombin activity with a standard.

15. (Original) The method of Claim 1, wherein the contact activator is selected from the group consisting of kaolin, clay, silica, ellagic acid, celite, diatomaceous earth, glass beads, and a combination thereof.

16. (Withdrawn and currently amended) A method of performing a clotting assay comprising:

- (a) combining in vitro a blood or plasma sample from a subject comprising blood or plasma with:
 - (i) a soluble-phospholipid that is soluble in the sample;
 - (ii) a contact activator; and
 - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for clot formation, thereby performing a clotting assay.

17. (Withdrawn) The method of Claim 16, further comprising determining a clotting time for the sample.

18. (Withdrawn) The method of Claim 17, further comprising comparing the determined clotting time with a standard.

19. (Withdrawn and currently amended) The method of Claim 16, wherein the sample is first combined with the contact activator, and is then combined with the soluble-phospholipid and the calcium to initiate the clotting reaction.

20. (Withdrawn and currently amended) The method of Claim 16, wherein the soluble-phospholipid consists essentially of a phospholipid selected from the group consisting of phosphatidylserine, phosphatidylhomoserine, phosphatidic acid, phosphatidylethanolamine, and a combination thereof.

21. (Withdrawn) The method of Claim 16, wherein the sample is a human blood or plasma sample.

22. (Withdrawn and currently amended) A method of detecting a deficiency in intrinsic clotting pathway activity comprising:

(a) combining in vitro a blood or plasma sample from a subject comprising blood or plasma with:

- (i) a soluble phospholipid that is soluble in the sample;
- (ii) a contact activator; and
- (iii) calcium;

(b) incubating the mixture of (a) above for a time and under conditions sufficient for clot formation;

(c) determining a clotting time for the sample;

(d) comparing the determined clotting time for the sample with a standard, wherein a prolonged clotting time as compared with the standard correlates with is indicative of a deficiency in intrinsic clotting pathway activity, thereby detecting a deficiency in intrinsic clotting pathway activity.

23-26 (Cancelled).

27. (Withdrawn and currently amended) A method of evaluating Factor VII_a activity comprising:

(a) combining in vitro a plasma sample from a subject comprising plasma with:

- (i) a soluble phospholipid that is soluble in the sample;
- (ii) soluble tissue factor; and
- (iii) calcium;

(b) incubating the mixture of (a) above for a time and under conditions sufficient for prothrombinthrombin activation; and

(c) detecting thrombin activity, wherein thrombin activity correlates with is indicative of Factor VIIa activity in the sample, thereby evaluating Factor VII_a activity.

28. (Withdrawn and currently amended) A method of evaluating the activity of a clotting factor in the intrinsic clotting pathway comprising:

(a) combining in vitro a plasma sample from a subject comprising plasma with:

- (i) a soluble phospholipid that is soluble in the sample;
- (ii) exogenous Factor X;
- (iii) activated phospholipid-dependent clotting factors other than Factor X that are dependent on the clotting factor being evaluated in the intrinsic clotting pathway or are required for activation of the clotting factor being evaluated; and
- (iii) calcium;

(b) incubating the mixture of (a) above for a time and under conditions sufficient for Factor X activation to Factor X_a; and

(c) detecting Factor X_a activity, wherein Factor X_a activity correlates with is indicative of the activity of the clotting factor in the sample, thereby evaluating the activity of a clotting factor in the intrinsic clotting pathway.

29. (Withdrawn) The method of Claim 28, wherein Factor X_a activity is detected by a spectrophotometric assay for Factor X_a.

30. (Withdrawn) The method of Claim 28, wherein Factor X_a activity is detected by detecting thrombin activity in the sample.

31. (Withdrawn) The method of Claim 28, wherein Factor X_a activity is detected by detecting clot formation.

32. (Withdrawn and currently amended) The method of Claim 28, wherein the method is carried out to evaluate FVIII_a activity, the method comprising:

- (a) combining in vitro ~~the~~ plasma sample comprising plasma with:
 - (i) ~~a soluble~~ ~~the~~ phospholipid that is soluble in the sample;
 - (ii) exogenous Factor X;
 - (iv) exogenous Factor IX_a; and
 - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for Factor X activation to Factor X_a; and
- (c) detecting Factor X_a activity, wherein Factor X_a activity correlates with ~~is indicative of~~ Factor VIII_a activity in the sample.

33. (Withdrawn) The method of Claim 32, wherein the sample is further combined with thrombin.

34-42. (Cancelled)

43. (Withdrawn and currently amended) A kit for evaluating clotting factor activity in a sample comprising:

- (a) a soluble phospholipid that is soluble in the sample; and
- (b) a reagent selected from the group consisting of (i) a contact activator, (ii) a soluble tissue factor, and (iii) a composition comprising Factor X.

44. (Withdrawn) The kit of Claim 43 further comprising calcium.

45. (Withdrawn) The kit of Claim 43 further comprising a standard.

46. (Withdrawn) The kit of Claim 43, wherein the kit comprises a contact activator and further comprises Activated Protein C or a Protein C activator.

47. (Withdrawn) The kit of Claim 46, wherein the kit further comprises Protein S depleted plasma.

48. (Withdrawn) The kit of Claim 43, wherein the kit comprises a composition comprising Factor X and a composition comprising Factor IX_a.

49. (Withdrawn) The kit of Claim 43, wherein the kit further comprises a plasma selected from the group consisting of (a) a plasma known to be deficient for a particular clotting factor and (b) normal plasma.

50. (Withdrawn and currently amended) The method of Claim 28, wherein the method is carried out to evaluate Factor IX_a activity, the method comprising:

- (a) combining in vitro thea plasma sample comprising plasma with:
 - (i) thea soluble phospholipid that is soluble in the sample;
 - (ii) exogenous Factor X;
 - (iv) exogenous Factor VIIIa or exogenous FVIII and a FVIII activator; and
 - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for Factor X activation to Factor X_a; and
- (c) detecting Factor X_a activity, wherein Factor X_a activity correlates with indicative of Factor IX_a activity in the sample.

51. (Withdrawn) The method of Claim 50, wherein the sample is further combined with thrombin.

52. (New and withdrawn) The method of Claim 16, wherein the sample is from a subject with lupus.

53. (New and withdrawn) The method of Claim 22, wherein the sample is from a subject with lupus.

54. (New and withdrawn) The method of Claim 27, wherein the sample is from a subject with lupus.

55. (New and withdrawn) The method of Claim 28, wherein the sample is from a subject with lupus.

56. (New and withdrawn) The method of Claim 32, wherein the sample is from a subject with lupus.

57. (New and withdrawn) The method of Claim 50, wherein the sample is from a subject with lupus.

58. (New) A method of evaluating clotting activity in a sample comprising:

- (a) combining *in vitro* a blood or plasma sample from a subject with:
 - (i) a phospholipid that is soluble in the sample to a final concentration of 50 μ M to 2 mM phospholipid;
 - (ii) a contact activator; and
 - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for prothrombin activation; and
- (c) detecting Factor X_a or thrombin activity, wherein the activity of Factor X_a or thrombin correlates with clotting factor activity in the sample, thereby evaluating clotting activity in the sample.

59. (New) The method of Claim 58, wherein the phospholipid is added to a final concentration of 200 μ M to 2 mM.

60. (New) The method of Claim 58, wherein the phospholipid comprises phospholipids acylated by C4 to C12 fatty acids.

61. (New) The method of Claim 58, wherein the sample is from a subject with lupus.

62. (New) A method of evaluating clotting activity in a sample comprising:

- (a) combining *in vitro* a blood or plasma sample from a subject with:
 - (i) a phospholipid that is soluble in the sample and contains no detectable aggregates as determined by quasi-electric light scattering techniques;
 - (ii) a contact activator; and
 - (iii) calcium;
- (b) incubating the mixture of (a) above for a time and under conditions sufficient for prothrombin activation; and
- (c) detecting Factor X_a or thrombin activity, wherein the activity of Factor X_a or thrombin correlates with clotting factor activity in the sample, thereby evaluating clotting activity in the sample.

63. (New) The method of Claim 62, wherein the phospholipid is added to a final concentration of 50 μ M to 2 mM.

64. (New) The method of Claim 62, wherein the phospholipid is added to a final concentration of 200 μ M to 2 mM.

65. (New) The method of Claim 62, wherein the phospholipid comprises phospholipids acylated by C4 to C12 fatty acids.

66. (New) The method of Claim 62, wherein the sample is from a subject with lupus.

67. (New) A method of evaluating clotting activity in a sample comprising:

- (a) combining *in vitro* a blood or plasma sample from a subject with:

- (i) a phospholipid that is soluble in the sample and consists essentially of phospholipids acylated by C2 to C14 fatty acids;
- (ii) a contact activator; and
- (iii) calcium;

- (b) incubating the mixture of (a) above for a time and under conditions sufficient for prothrombin activation; and
- (c) detecting Factor X_a or thrombin activity, wherein the activity of Factor X_a or thrombin correlates with clotting factor activity in the sample, thereby evaluating clotting activity in the sample.

68. (New) The method of Claim 67, wherein the phospholipid is added to a final concentration of 50 μ M to 2 mM.

69. (New) The method of Claim 67, wherein the phospholipid is added to a final concentration of 200 μ M to 2 mM.

70. (New) The method of Claim 67, wherein the phospholipid consists essentially of phospholipids acylated by C4 to C10 fatty acids.

71. (New) The method of Claim 67, wherein the sample is from a subject with lupus.